Sulfite Agar

Intended Use

Sulfite Agar is used for detecting thermophilic, H_2S-producing anaerobes, particularly in foods.

Summary and Explanation

Sulfide spoilage of foods is due to three factors: high spore counts, the heat resistance of the spores and subjecting the finished product to elevated temperatures. The last factor may occur if the processed food is not cooled adequately.[1]

Clark and Tanner[2] described the thermophilic organisms that cause spoilage in canned foods as flat-sour spoilage organisms, thermophilic anaerobes and sulfide-spoilage organisms. They used Sulfite Agar to study sulfide-spoilage organisms in sugar and starch.

Both beet and cane sugar can carry spores of the thermophilic bacteria that are spoilage agents.[3] *Desulfotomaculum nigrificans*, first classified as *Clostridium nigrificans*, causes spoilage in non-acid canned foods such as vegetables and infant formula.[1] The growth of *D. nigrificans* occurs in the range of pH 6.2-7.8, with the best growth occurring at pH 6.8-7.3. Scanty growth can be observed at pH 5.6. The reaction of most vegetables, except corn and peas, falls below pH 5.8, so sulfide spoilage is rare.[1]

Sulfite Agar is a recommended standard methods medium for detecting sulfide spoilage bacteria.[1,3]

Principles of the Procedure

Sulfite Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium sulfite, upon reduction, produces hydrogen sulfide. Agar is the solidifying agent.

Iron nails or iron strips will combine with any dissolved oxygen in the medium and provide an anaerobic environment.

Formula

Difco™ Sulfite Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	10.0	g
Sodium Sulfite	1.0	g
Agar	20.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Sulfite Agar

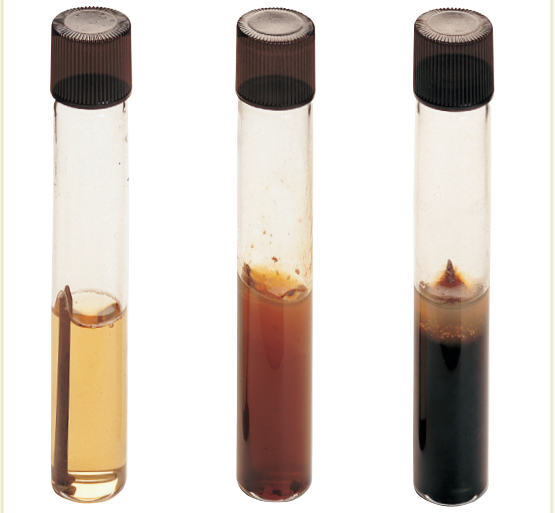
Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Difco™ Sulfite Agar

Prepare the medium per label directions. Inoculate molten medium, solidify and incubate aerobically at 55 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	SULFITE REDUCTION
Bacillus stearothermophilus	10149	30-100	Good	–
Clostridium thermosaccharolyticum	7956	30-100	Good	+
Desulfotomaculum nigrificans	19858	30-100	Good	+
		Uninoculated		



Tube

Bacillus

stearothermophilus ATCC™ 10149

nigrificans ATCC™ 19858

Desulfotomaculum

Procedure[1]

Sugar

1. Place 20 g of dry sugar in a dry, sterile, graduated 250 mL Erlenmeyer flask closed with a rubber stopper.
2. Add sterile water to the 100 mL mark and shake to dissolve.
3. Replace the stopper with a sterile cotton plug, bring the solution rapidly to a boil, and continue boiling for 5 minutes.
4. Replace evaporated liquid with sterile water.
5. Cool immediately in cold water.

NOTE: For liquid sugar, prepare as for dry sugar, except determine the amount of liquid sugar needed on the basis of degree Brix in order to be equivalent to 20 g of dry sugar.[3]

6. Divide 20 mL of heated sugar solution among 6 screw-cap tubes (20 × 150 mm) containing approximately 10 mL of freshly autoclaved, still molten Sulfite Agar and a nail.
7. Make the inoculations into freshly autoclaved medium, and cool and solidify immediately in cold water.
8. Preheat the tubes to 50-55°C.
9. Incubate at 50-55°C for 24-48 hours.

Starch and Flour

1. Place 20 g of starch or flour in a dry, sterile, graduated 250 mL Erlenmeyer flask.
2. Add sterile water to the 100 mL mark, swirling occasionally.
3. Close the flask with a sterile rubber stopper.
4. Shake well to obtain a uniform, lump-free suspension. Add sterile glass beads to the sample mixture to aid in thoroughly mixing during shaking.
5. Divide 20 mL of the starch or flour suspension among 6 screw-cap tubes (20 × 150 mm) containing approximately 10 mL of freshly autoclaved, still molten Sulfite Agar and a nail.
6. Swirl the tubes several times to ensure even dispersion of the starch or flour in the medium. Heat in a boiling water bath for 15 minutes, continuing to swirl the tubes.
7. Cool and solidify immediately in cold water.
8. Preheat the tubes to 50-55°C.
9. Incubate at 50-55°C for 24-48 hours.

Nonfat Dry Milk

1. Place 10 g of nonfat dry milk in a sterile, graduated 250 mL Erlenmeyer flask.
2. Add .02N sodium hydroxide to the 100 mL mark.
3. Shake to completely dissolve.
4. Autoclave at 5 pounds pressure for 10 minutes.
5. Cool immediately.
6. Transfer 2 mL of nonfat dry milk solution to each of two screw-cap tubes (20 × 150 mm) containing freshly autoclaved, still molten Sulfite Agar and a nail.
7. Gently swirl several times.
8. Cool and solidify immediately in cold water.
9. Preheat the tubes to 50-55°C.
10. Incubate at 50-55°C for 24-48 ± 3 hours.

Cream

1. Mix 2 g of gum tragacanth and 1 g of gum arabic in 100 mL of water in an Erlenmeyer flask.
2. Autoclave at 121°C for 20 minutes.
3. Transfer 20 mL of cream sample to a sterile, graduated 250 mL Erlenmeyer flask.
4. Add sterilized gum mixture to the 100 mL mark.
5. Shake carefully using a sterile rubber stopper.
6. Loosen the stopper. Autoclave at 5 pounds pressure for 5 minutes.

Soy Protein Isolates

1. Prepare a 10% suspension of soy protein isolate in sterile 0.1% peptone water in milk dilution or similar bottles.
2. Adjust to pH 7.0 ± 0.1.
3. Steam in an autoclave at 5 pounds pressure for 20 minutes.
4. Add 1 mL of soy protein isolate suspension to each of 10 tubes containing freshly autoclaved, still molten Sulfite Agar and a nail. If using already prepared medium, heat the tubes immediately before inoculation to eliminate oxygen.
5. Mix tubes.
6. Solidify in an ice water bath.
7. Overlay with vaspar (one part mineral oil combined with two parts petroleum jelly, heated in an oven at 191°C for 3 hours).
8. Preheat the tubes to 55°C.
9. Incubate at 55°C for 14 days. Take preliminary counts at 48 hours, 7 days and 14 days in case tubes become completely blackened.
10. Count the blackened areas for each tube and report as the number of spores per gram of soy isolate.

Expected Results

Hydrogen sulfide production from the reduction of sulfite causes a blackening of the medium.

Sulfide spoilage spores should be present in not more than two of five samples tested (40%) with not more than 5 spores per 10 g in any one sample.[1]

Limitations of the Procedure

1. Nails or iron strips should be cleaned in hydrochloric acid and rinsed well to remove any rust before being placed into tubes of medium.
2. If iron nails or iron strips are not available, substitute 10 mL of 5% ferric citrate solution.
3. Spoiled peas may not show discoloration but will show blackening with a dark-colored brine.
4. Spangling of the enamel may occur as a result of the interaction of dissolved hydrogen sulfide with the iron of the container.

References

1. Donnelly and Hannah. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Clark and Tanner. 1937. Food Res. 2:27.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Sulfite Agar

AOAC COMPF

Cat. No. 297210 Dehydrated – 500 g

BD